REMARKS-General

Upon review of the original and previously amended specifications and claims, and also in light of the observation of the Examiner noted in the above Office Action, the applicants have further amended the current claims as described above. No new matter has been included. The purpose of the current amendments to Claims 1, 2, 22 and 23 is to clarify the limitations of the innovative processes for amplifying sense-oriented and full-length version of the mRNAs and resulting in novel composition matter of sense-oriented full-length RNA amplicons of the Present Invention.

A formal Information Disclosure Statement and copies of related arts are submitted to be of sufficient clarity and detail to enable a person of average skill in the art to make and use the instant invention, so as to be pursuant to 35 USC 112. Accordingly, References 1 to 4 are relevant publication documents to disclose the non-feature subject matters to be claimed in the instant invention.

Response to Rejection of Claims 1-3, 7-18, 20, 22, 23, 25, 26 and 29-35 under 35USC112

According to the guidance in *In re* Wands, 8 USPQ2d 1400 (CAFC 1988), the applicants respectfully submit that the examined claims 1, 2, 22 and 23 are amended to fully match the claimed subject matter of the instant invention to the description of the original specifications and examples, as pursuant to 35USC112, first paragraph.

The amended Claim 1 clarifies that the instant invention is an mRNA amplification process with full-length capacity up to 4.9 kilo-bases.

The amended Claim 22 clarifies that the instant invention is an improved mRNA amplification process with improvement capacity up to 4.9 kilo-bases.

In response to Points 4-9 and 18 of the Examiner's rejection, the applicants respectively correct the Claims 1, 2, 22 and 23, providing clear limitations in the full-length products and cycles of amplification of the present invention.

The size limitation is described in the FIG 4 and Example 4 of the present invention.

The applicants agree that the present invention is limited by an average of 4% deviation rate in each cycle of amplification, as recently shown in the Figure 2 of the **Reference 1** (Ying et al., *Bioch. Biophs. Res. Comm.* 313:104, 2004). This minor deviation is not statistically significant in a one- or two-cycle amplification process; however, the accumulative effect of multiple deviations after three-cycle amplification will become significant (> 10%). It is noted that truncated cDNAs were quickly lost during cycling of the amplification process. Currently, no evidence clearly indicates the cause of this deviation. Thus, the Claims 2 and 23 are deleted.

In response to Points 10-13 of the Examiner's rejection, the applicants have clearly described the use of phage T7 and SP6 RNA promoters in the Specification and Examples 3, 4 and 5 of the present invention. As published by Sastry et al. in 1999 (*Biochemistry* 38:4972; **Reference 2**), it teaches "*T7 RNA polymerase is.....unlike the eukaryotic and bacterial RNA polymerases, it is a single subunit enzyme and does not require additional factors to carry out the entire process of transcription from start to finish. .....in the T7 RNA polymerase-promoter complex, the polymerase molecule mainly contacts the template bases in the TATA box while the upstream contacts are used as an anchor for DNA binding.*" Thus, it states that the claimed phage RNA polymerase does not required any transcription factor and also the TATA box is just one part of the phage RNA promoter. Because an RNA promoter contains all essential parts of transcriptional initiation sequences, such as anchoring site, melting domain and TATA-like box, the applicants believe that the term of "RNA promoter" is more complete and appropriate than that of "TATA box" in the description of the present invention.

The difficulties described in the US Patent Publication 2003/0087275 are directed to an eukaryotic RNA polymerase system, *e.g.* type II RNA polymerases in yeasts and mammals, which are very different from the claimed phage systems, such as T7, T3 and SP6. The argument based on such differently systemic conditions is inappropriate and irrelevant to the present invention. As emphasis in the US Patent Publication 2003/0087275, it teaches "*The TATA box or the like, which may be comprised in the DNA sequence for regulating transcription of the invention can be*

found in various species ranging from simple eukaryotes such as baker's yeast to more complex organisms such filamentous fungi and humans." This description clearly states an eukaryotic system and further excludes the prokaryotic and phage systems from the range of yeast to humans. For *in vitro* transcription, T7, T3 and SP6 RNA polymerases are widely used and it is well known that T7, T3 and SP6 RNA promoters all contain a TATA-like box in their sequences as described by the Reference 2. The applicants have described the use of the phage RNA promoter-primers, such as oligo(dT)-T7 and oligo(dC)-SP6, which already include a TATA-like box in their promoter regions. Thus, there is no need to add an extra TATA box in the internal cDNA region or to add any transcription factor for the transcriptional processing of the present invention.

In response to Point 14-27 of Examiner's rejection, the applicants respectively believe that the problems of enzyme fidelity and secondary RNA structure are out of scope of the present invention. These problems do cause early truncation of cDNA products during reverse transcription; however, none of these truncated cDNAs can reach the 5'-ends of reverse-transcribed mRNAs for the tailing reaction, as shown in the step (c) of Claim 1 and step (b) of Claim 22. Without completion of the tailing reaction, the truncated cDNAs will be excluded from the procedure of the present invention without any amplification. The requirement of an exposed 3'-end for efficient DNA tailing has already been demonstrated by Michelson et al. in 1982 (*J. Biol. Chem.* 257:14773, **Reference 3**), it teaches "*The protruding 3'-termini... were more efficiently utilized by terminal transferase, ...In contrast, it was assumed that blunt or 3'-recessed ends offers less accessible sites for terminal transferase binding. As a result, ...These findings were consistent with earlier work which demonstrated that terminal transferase requires a single-stranded initiator having a chain length of at least three deoxynucleotide residues.*" Thus, the truncated cDNAs caused by the problems of enzyme infidelity and secondary RNA structure will never produce 3'-exposing deoxynucleotide termini, which are required for the tailing reaction. Only the full-length cDNAs that reach the ends of the template mRNAs can be tailed by terminal transferase and then continue the full process of the present invention. This point clearly indicates the full-length amplification property of the present invention.

The above explanation also states that the cDNA truncation caused by enzyme infidelity and/or inefficiency in the US Patent Publication 2003/0040099 and US Patents

5,804,380, 6,140,053, 6,303,306, 6,361,940 and 5,858,671 has been prevented by the tailing procedure of the present invention. Because the applicants agree that there are certain levels of infidelity and inaccuracy in all kinds of enzymatic reactions, the present invention never claim for the existence of an enzymatic process with 100% fidelity and accuracy, which is out of the scope of the present invention. To this end, the applicants believe that the current amendment has provided more limitations to the present invention in order to prevent potential mis-understanding of the claims.

Because only the full-length cDNAs which reach the ends of the template mRNAs during reverse transcription can be tailed by terminal transferase and then continue the full process of the present invention, the difficulties of secondary RNA structure in the US Patent Publication 2003/0180737 and US Patents 5,858,671 have been overcome by the tailing procedure of the present invention. As shown in the Figure 2 of the Reference 1, Ying et al. have proven that the present invention is able to generate an amplified RNA libraries with a correlation coefficiency rate more than 96%, suggesting that the truncated cDNA ratio is about 4% of the original RNA population, which is lost during amplification and is not statistically significant. Since the present invention has excluded the truncated cDNAs from its amplified products, the problem of secondary RNA structure is out of the scope of the present invention. The present invention does not claim for a process to amplify all RNA species in an mRNA library. Currently, no evidence clearly indicates which species of mRNAs tend to be truncated during reverse transcription.

In response to Points 15, 16, 25, and 26 of Examiner's rejection, new evidence has been found to support the fluorescein-labeled RNA production by RNA polymerase. As published by Egger et al. in 1999 (*Histochem. Cell Biol.* 111:319; **Reference 4**), it teaches "*.....strand-specific single-stranded RNA probes labeled directly with the fluorochromes fluorescein isothiocyanate or Texas red. The probes, produced by in vitro transcription from PCR-generated templates with T7 RNA polymerase and fluorochromized UTP, gave ISH signals directly visible by fluorescence microscopy....*". Based on this evidence, the disclosure of undue experimentation for the use of modified nucleotides by RNA polymerases is fulfilled.

In summary, several new evidences (References 1-4) suggest that the prior arts, as speculated by the Examiner, are out of the scope of the Present Invention. First, the described eukaryotic system of US Patent Publications 2003/0087275 is incomparable to the phage system of the present invention. The difficulties of secondary RNA structures found in the US Patent Publication 2003/0180737 and US Patents 5,858,671 have been excluded by the tailing procedure of the present invention. Further, the problems of enzyme infidelity in the US Patent Publication 2003/0040099 and US Patents 5,804,380, 6,140,053, 6,303,306, 6,361,940 and 5,858,671 are common to all enzymatic reactions and the present invention does not claim for an enzymatic process with 100% accuracy, which is out of the scope of the present invention. Therefore, the basis for decision of claim rejection to Claims 1, 3, 7-18, 20, 22, 25, 26 and 29-35 under 35 U.S.C. 112, first paragraph, was unfounded. The applicants believe that the current amendment has provided more limitations to the present invention in order to clarify the claims. No new matter is added.

The applicants believe that for all of the foregoing reasons, all of the Claims 1, 3, 7-18, 20, 22, 25, 26 and 29-35 are in condition for allowance and such action is respectfully requested.

The Cited but Non-Applied References

The cited but not relied upon references have been studied and are greatly appreciated, but are deemed to be less relevant than the relied upon references.

In view of the above, it is submitted that the claims are in condition for allowance. Reconsideration and withdrawal of the objection are requested. Allowance of claims 1, 3, 7-18, 20, 22, 25, 26 and 29-35 at an early date is solicited.

Should the Examiner believe that anything further is needed in order to place the application in condition for allowance, he is requested to contact the undersigned at the telephone number listed below.

Respectfully submitted,

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